

Supplementary Material

1 Supplementary Tables

Supplementary Table 1. Nutritional composition of grape polyphenol-soy protein isolate (GP-SPI) complex and SPI (per 100 g)

Diet	SPI	GP-SPI
Calories	386	392
Calories from fat	29	40
Ash (%)	3.9	4.5
Moisture (%)	4.6	3.0
Carbohydrates (%)	0	31.3
Total Dietary Fiber	< 0.1	21.0
Protein (%)	89.3	56.7
Total Fat (%)	3.24	4.4
Sugars (%)		
Glucose	< 0.1	1.15
Fructose	< 0.1	1.58
Sucrose	< 0.1	0.35
Total Sugars (%)	0	3.08

Supplementary Table 2. Mouse diet formulations

	LFD	WD	WD-GP
Ingredient (g)			
Casein	75.5	95.5	95.5
Soy Protein, Supro 661	0	0	36.3
DL – Methionine	3	3	3
GP-SPI	0	0	96.8
Soy Protein	116.5	96.8	0
Corn Starch	641.9	0	0
Maltodextrin 10	150	99.1	91.6
Sucrose	0.4	340.3	340
Dextrose	1.1	0.9	0
Fructose	1.6	1.3	0
Cellulose	50	50	29.7
Butter	42.5	200	200
Corn Oil	30	30	27.3
Cholesterol	0	1.5	1.5
Ethoxyquin	0.04	0.04	0.04
Mineral Mix S10001	35	35	35
Calcium Carbonate	4	4	4
Vitamin Mix V10001	10	10	10
Choline Bitartrate	2	2	2
Red Dye #40, FD&C	0	0	0.025
Blue Dye #1, FD&C	0.05	0.025	0
Yellow Dye #5, FD&C	0	0.025	0.025
Total (g)	1163.59	969.49	972.79
Kcal%			
Protein	15	15	15
Carbohydrate	70	39	39
Dextrose	0.1	0.1	0.1
Sucrose	1	30	30
Fructose	0.1	0.1	0.1
Fat	15	46	46
Total	100	100	100
Kcal/gm	3.96	4.75	4.73
kJ/gm	16.58	19.90	19.84

Supplementary Table 3. Composition of dairy butter based used in LFD, WD, and WD-GP-diets, and of lard used in our previous studies, based on manufacturer data

Ingredient	Butter, Anhydrous gm	Lard
C2, Acetic	0.0	0.0
C4, Butyric	3.2	0.0
C6, Caproic	1.9	0.0
C8, Caprylic	1.1	0.0
C10, Capric	2.5	0.1
C12, Lauric	2.8	0.1
C14, Myristic	10.0	1.2
C14:1, Myristoleic	1.5	0.0
C15	N/A	0.1
C16, Palmitic	N/A	20.0
C16:1, Palmitoleic	2.3	1.5
C17	N/A	0.3
C18, Stearic	12.1	11.0
C18:1, Oleic	25.1	34.9
C18:2, Linoleic	2.3	21.9
C18:3, Linolenic	1.4	1.3
C18:4, Stearidonic	0.0	0.0
C20, Arachidic	1.0	0.2
C20:1	0.0	0.6
C20:2	0.0	0.8
C20:3	0.0	0.1
C20:4, Arachidonic	0.0	0.3
C20:5, Eicosapentaenoic	0.0	0.0
C21:5	0.0	0.0
C22, Behenic	0.0	0.0
C22:1, Erucic	0.0	0.0
C22:4, Clupanodonic	0.0	0.0
C22:5, Docosapentaenoic	0.0	0.1
C22:6, Docosahexaenoic	0.0	0.0
C24, Lignoceric	0.0	0.0
C24:1	0.0	0.0
Total	93.4	94.3
Saturated (g)	60.8	32.9
Monounsaturated (g)	28.9	37.0
Polyunsaturated (g)	3.7	24.4
Saturated (%)	65.1	34.9
Monounsaturated (%)	31.0	39.2
Polyunsaturated (%)	4.0	25.9

Supplementary Table 4. Taqman assays

TaqMan primer	Assay ID
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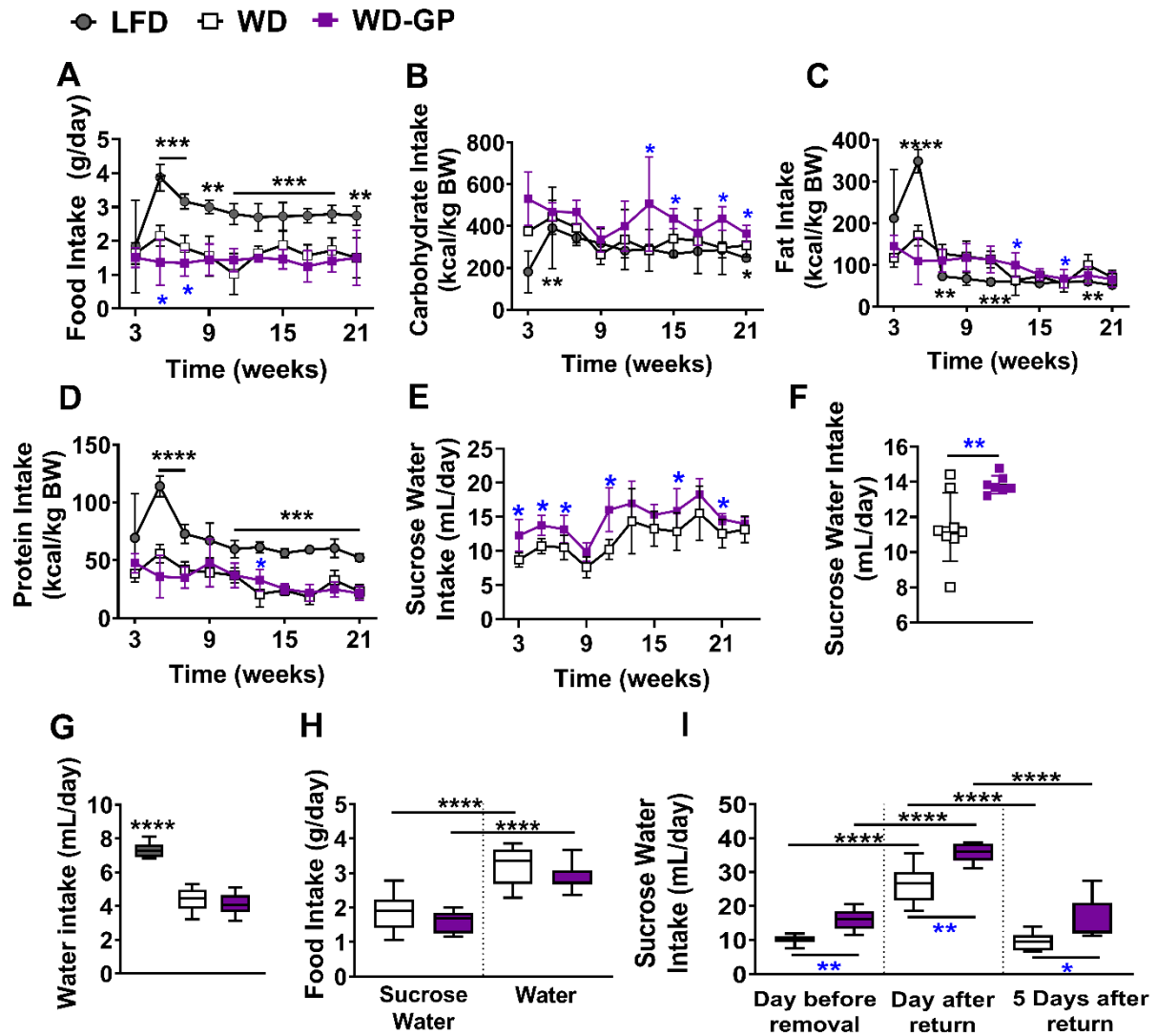
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<i>Gcg</i>	Mm00801714_m1
<i>HK3</i>	Mm01341942_m1
<i>HMBS</i>	Mm01143545_m1
<i>KHK</i>	Mm00434647_m1
<i>LDHa</i>	Mm01612132_g1
<i>LDHb</i>	Mm05726463_g1
<i>Pcsk1</i>	Mm00479023_m1
<i>Ppargc1a</i>	Mm01208835_m1
<i>SLC16A1</i>	Mm01306379_m1
<i>PDHx</i>	Mm00558275_m1
<i>RPLP0</i>	Mm00725448_s1
<i>SLC5A1</i>	Mm00451203_m1
<i>SLC2A2</i>	Mm00446229_m1
<i>SLC2A5</i>	Mm00600311_m1
<i>UCP1</i>	Mm01244861_m1

Supplementary Table 5. Gene Expression Data

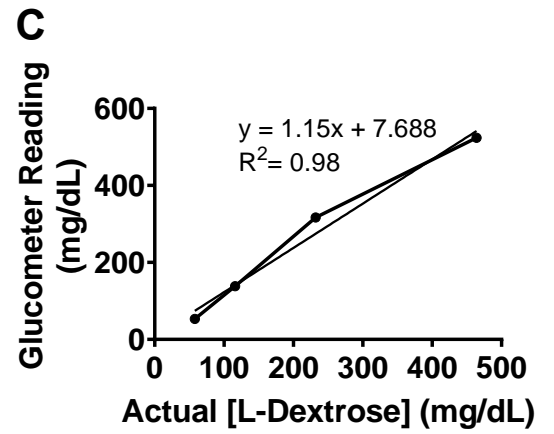
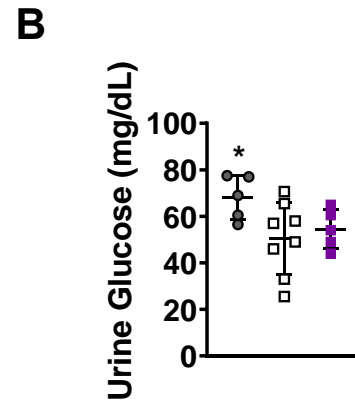
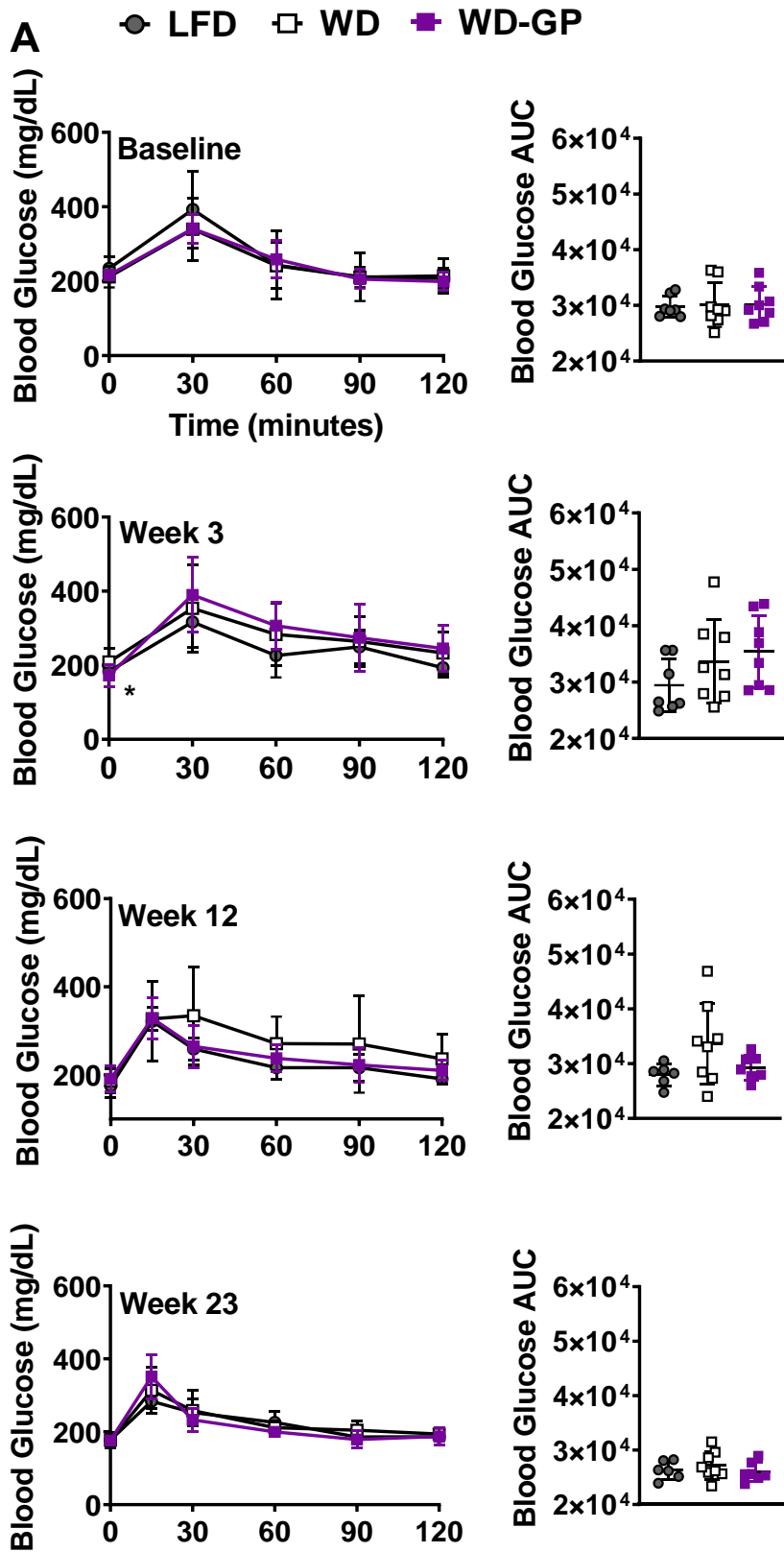
Tissue	Gene	WD-GP (n= 5 – 8)	WD (n= 6 – 8)	p-value	Sig.
Liver	<i>CPT1a</i>	5.79 ± 0.93	6.67 ± 1.87	0.30	ns
	<i>FAS</i>	0.17 ± 0.02	0.2 ± 0.07	0.29	ns
	<i>FGF21</i>	0.40 ± 0.035	0.37 ± 0.40	0.80	ns
	<i>G6PC</i>	3.35 ± 2.42	3.13 ± 1.99	0.87	ns
	<i>HK3</i>	0.02 ± 0.01	0.02 ± 0.006	0.96	ns
	<i>KHK</i>	7.18 ± 1.11	10.95 ± 5.32	0.11	ns
	<i>SLC16A1; MCT-1</i>	1.27 ± 0.66	1.09 ± 0.2	0.55	ns
	<i>SLC2A2; GLUT2</i>	4.98 ± 2.02	5.00 ± 0.59	0.98	ns
	<i>SLC2A5; GLUT5</i>	0.31 ± 0.16	0.39 ± 0.17	0.40	ns
Brown adipose	<i>UCP1</i>	185.6 ± 76.45	240.7 ± 58.75	0.18	ns
	<i>Ppargc1a</i>	0.75 ± 0.22	0.57 ± 0.09	0.11	ns
Duodenum	<i>G6PC</i>	5.3 ± 2.01	9.04 ± 4.87	0.09	ns
	<i>Gcg</i>	0.65 ± 0.19	0.72 ± 0.25	0.57	ns
	<i>HK3</i>	0.023 ± 0.01	0.019 ± 0.006	0.48	ns
	<i>KHK</i>	19.19 ± 5.96	23.67 ± 10.26	0.35	ns
	<i>LDHa</i>	23.72 ± 3.83	24.07 ± 3.45	0.86	ns
	<i>LDHb</i>	0.29 ± 0.13	0.36 ± 0.15	0.32	ns
	<i>PDHx</i>	1.1 ± 0.18	0.82 ± 0.21	0.02	*
	<i>Pcsk1</i>	0.07 ± 0.01	0.09 ± 0.02	0.06	ns
	<i>SLC16A1; MCT-1</i>	4.01 ± 1.05	3.43 ± 0.59	0.23	ns
	<i>SLC5A1 SGLT1</i>	88.07 ± 40.36	86.47 ± 34.17	0.94	ns
	<i>SLC2A2; GLUT2</i>	26.22 ± 8.74	23.57 ± 7.84	0.55	ns
	<i>SLC2A5; GLUT5</i>	81.18 ± 17.04	78.96 ± 27.51	0.85	ns
	Jejunum	<i>G6Pc</i>	1.93 ± 1.48	2.79 ± 1.44	0.33
<i>HK3</i>		0.009 ± 0.002	0.008 ± 0.003	0.23	ns
<i>KHK</i>		11.82 ± 3.47	14.5 ± 3.51	0.21	ns
<i>LDHa</i>		14.24 ± 8.44	30.19 ± 16.78	0.05	*
<i>LDHb</i>		0.041 ± 0.007	0.039 ± 0.02	0.87	ns
<i>PDHx</i>		0.47 ± 0.11	0.43 ± 0.09	0.49	ns
<i>SLC16A1; MCT-1</i>		2.73 ± 0.53	3.09 ± 0.42	0.23	ns
<i>SLC5A1 SGLT1</i>		50.58 ± 13.28	53.04 ± 25.28	0.82	ns
<i>SLC2A2; GLUT2</i>		15.5 ± 2.62	16.72 ± 5.61	0.61	ns
<i>SLC2A5; GLUT5</i>		19.41 ± 5.05	24.54 ± 6.44	0.14	ns
Ileum		<i>G6PC</i>	4.19 ± 4.87	0.49 ± 0.60	0.12
	<i>HK3</i>	0.19 ± 0.09	0.08 ± 0.09	0.06	ns
	<i>KHK</i>	62.36 ± 43.71	13.11 ± 15.08	0.04	*
	<i>LDHa</i>	227.9 ± 214.1	262.9 ± 150.9	0.75	ns
	<i>LDHb</i>	5.31 ± 3.25	4.79 ± 3.62	0.79	ns
	<i>PDHx</i>	4.95 ± 3.86	9.09 ± 6.94	0.23	ns
	<i>SLC16A1; MCT-1</i>	32.96 ± 28.98	34.87 ± 18.76	0.89	ns
	<i>SLC2A2; GLUT2</i>	65.71 ± 56.17	7.52 ± 9.12	0.05	*
	<i>SLC2A5; GLUT5</i>	65.13 ± 67.53	10.92 ± 12.72	0.11	ns
Colon	<i>Gcg</i>	5.54 ± 2.39	5.07 ± 3.09	0.76	ns
	<i>LDHa</i>	13.96 ± 14.05	21.47 ± 16.24	0.35	ns
	<i>LDHb</i>	1.22 ± 0.62	0.79 ± 0.49	0.17	ns
	<i>Pcsk1</i>	11 ± 2.79	14.39 ± 4.97	0.13	ns
	<i>SLC16A1; MCT-1</i>	1.85 ± 1.42	8.76 ± 4.88	0.005	**

Means and statistical differences between WD and WD-GP mRNA levels determined by unpaired, two-tailed student t-test with Welch's correction if group numbers differed. Data are shown as mean ± SD. *, p<0.05, **p<0.01. Sig.: significance. ns: not significant.

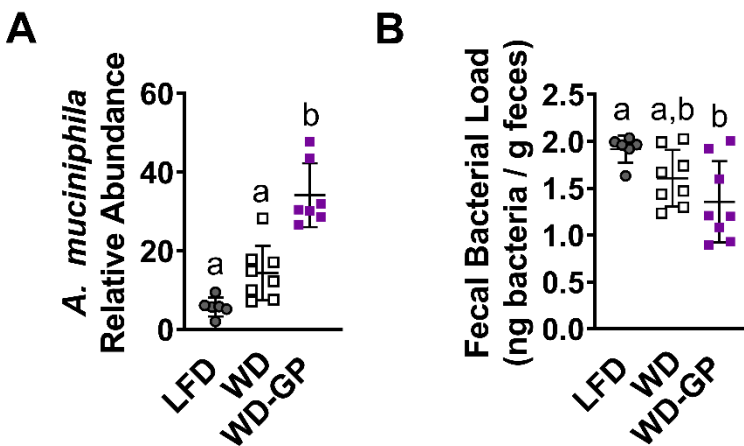
2 Supplementary Figures



Supplementary Figure 1. GPs increased caloric intake from sucrose water but not food. (A) Mean daily food consumption (g/day) at indicated week of diet intervention. Caloric intake per kg of BW from (B) carbohydrates, (C) fats, and (D) protein over the diet-intervention (n=6-8/group). (E) mean daily consumption of sucrose water (mL/day) at indicated week of diet intervention period, and (F) daily sucrose water consumption averaged from 23 weeks of diet intervention. (G) Mean water consumption after sucrose water was removed for two days. (n= 6-8/group). (H) Food intake during consumption of sucrose water vs. tap water (n= 6-8/group). (I) Sucrose water consumption (mL/day) the day before removal and replacement with plain water, one day after sucrose water was returned, and 5 days after sucrose water was returned. Data are presented as mean \pm SD. Significant difference between three groups was determined by one-way ANOVA followed by Dunnett's multiple comparisons test using the WD group as control. Black colored asterisks indicate statistical significance between the LFD vs WD group and blue-colored asterisks indicate statistical significance between the WD vs WD-GP group. In panels E, F, H, and I, an unpaired, two-tailed, t-test with Welch's correction was used; *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001. BW: Body Weight.



Supplementary Figure 2. Butter-based WD did not promote hyperglycemia. (A) Oral glucose tolerance test (OGTT) results at baseline and after 3, 12 and 23 weeks of diet-intervention. Left panels: blood glucose over the 120-minutes. Right panels: mean area under the curve (AUC) data from individual mice. (B) Unfasted urinary glucose levels measured using a glucometer. (C) Correlation of glucose concentration measured by glucometer vs. dilutions of L-dextrose used for standard curve providing validation for use of glucometer to measure urinary glucose. Data are presented as mean \pm SD. Significant differences between diet-group was determined by one-way ANOVA followed by Dunnett's multiple comparisons test using the WD group as control; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.



Supplementary Figure 3. GP supplementation induced a bloom of *Akkermansia muciniphila*.

(A) Abundance of *A. muciniphila* in fecal samples relative to total bacteria and archaea, and (B) total fecal bacterial and archaeal load at week 8 of diet-intervention. Data are presented as mean \pm SD. Different letters denote significant differences between diet-groups as determined by one-way ANOVA followed by Tukey's multiple comparisons test.

